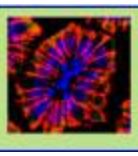

Science Advances from NIDDK

Advances for Winter 2010

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New Genetic Variants Associated with Celiac Disease Are Identified

Scientists have uncovered new genetic variants that are associated with the risk celiac disease and have linked these variants to four pathways of the immune system. Celiac disease is a complex genetic disease that can cause damage to the intestine, resulting in poor absorption of nutrients, painful digestive and other symptoms, and other serious complications. These symptoms occur when people with the disease eat grains containing gluten--such as from wheat, rye, and barley--which provokes an abnormal immune response that attacks their intestine. For children, celiac disease can have devastating consequences, such as impaired growth and development, while adults may experience anemia, bone loss, and other complications.

In an earlier study, scientists conducted a genome-wide association study (GWAS) to identify two gene variants that are required for celiac disease, and 12 chromosome regions that are associated with a risk for the disease. Although these findings were impressive, it was determined that all of the known variants did not account entirely for the genetic risk of celiac disease. In the new study, scientists set out to identify variants that may have smaller, yet critical, effects on disease risk. This was accomplished with a larger GWAS that included DNA samples from a larger number of patients with celiac disease and healthy volunteers. The samples were analyzed using a denser concentration of probes to identify differences in the DNA sequences of the patients compared with those of the volunteers. This approach was successful in uncovering 13 new chromosome regions that are associated with celiac disease and 13 additional chromosome regions with suggestive associations with celiac disease. Many of these regions were found to contain genes with functions related to the immune system. In addition, uncovering the genetic variants led the scientists to identify four specific immunological pathways that are relevant to the pathogenesis of celiac disease. The scientists also found that more than half of the variants associated with celiac disease correlate with the extent to which nearby genes are turned on or turned off (expressed), indicating that the variants may increase the risk of celiac disease by influencing the expression of other genes. These new findings have advanced knowledge of celiac disease and may also have important implications for other autoimmune diseases, such as type 1 diabetes.

Dubois PC, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. Nat Genet 42: 295-302, 2010.

Immune Cell Surface Protein Links Gut Bacteria to Diet and Protection Against Inflammation

Microbes residing throughout the human body are now being appreciated for their contributions both to human health and disease. Scientists have shown that the community of microbes living in the human gut have "co-evolved" with their host to the point that a well-balanced gut microbial community is essential for healthy functioning of the digestive system, as well as the immune system, to prevent conditions such as inflammatory bowel diseases (IBD) and other inflammatory or autoimmune conditions. For example, gut microbes perform many functions that humans are incapable of, such as harvesting certain nutrients from the foods we consume. Some bacterial species break down dietary fiber into short-chain fatty acids in the large bowel. These fatty acids have been shown to have a beneficial, anti-inflammatory effect on conditions such as IBD, and are known to act directly on cells in the gut or elsewhere by latching onto components of the cell's surface known as "receptors," including one called GPR43 in humans, or Gpr43 in mice.

In this study, researchers aimed to identify the mechanism by which this type of fatty acids, produced as a result of bacterial fermentation of dietary fiber, have a protective effect against IBD and other inflammatory conditions, such as arthritis and asthma. They showed that the presence of intestinal bacteria reduces disease severity using a mouse model that mimics a form of IBD known as ulcerative colitis. For this experiment, they compared mice raised conventionally with those raised in a bacteria-free environment, before and after their guts were repopulated with bacteria. Based on prior knowledge of microbial effects on IBD via production of short-chain fatty acids, which act through receptors like GPR43, the scientists utilized microarray screening technology to identify immune cells that produce high amounts of GPR43/Gpr43 in humans and mice, respectively. In mice genetically engineered to lack Gpr43 and treated to model ulcerative colitis, immune cells did not respond normally to short-chain fatty acids, and these fatty acids did not reduce intestinal inflammation as in wild-type mice. The results of this experiment indicate that short-chain fatty acids act through Gpr43 on the surface of immune cells to exert their protective effect against intestinal inflammation. Similar results were seen in mouse models of arthritis and allergic airway inflammation.

This study identifies how interactions between by-products of bacterial metabolism and a receptor on the surface of immune cells act to protect against IBD and other inflammatory conditions, such as rheumatoid arthritis and allergic inflammation of the airways. These interactions could provide a target for manipulating immune responses in these conditions through such means as diet and prebiotic/probiotic supplementation.

Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461:1282-1286, 2009.

The Human Microbiome – Bacterial "Census" Reveals that Healthy People Host Distinct Communities of Microbes

Researchers have developed a catalogue of the diversity and variation in bacterial species that reside on or within the body of healthy people. The human body is host to an enormous ecosystem of microorganisms. This microbial community—or microbiota—contains nearly 100 trillion organisms, with the number of bacterial cells on or in the human body outnumbering human cells by almost ten to one. The resident bacterial communities provide important functions that aid in metabolism, help prevent infections, and train the immune system. Since these traits are critical for normal health and may, if altered, contribute to disease, it is important to understand the diversity and variation of the human microbiota at different body sites, among individuals, and over time. Although previous studies revealed the diversity of the bacterial communities residing at distinct body sites, an integrated view of the human microbiota across the entire body is needed to fully define the genetic diversity that contributes to normal health.

Using state-of-the-art DNA sequencing methods, researchers have taken a census of the bacterial communities across several body sites of healthy individuals. The researchers collected samples of the bacterial communities from different body sites—including the gut, mouth, ears, nose, hair, and various skin surfaces—of healthy volunteers on several occasions over a three month period. After isolating the bacterial DNA from these samples, the scientists analyzed the DNA sequences of a particular gene known to vary among different bacterial species to determine the diversity of bacterial species present at different sites for each individual. They found that the composition of the bacterial communities was determined mostly by their location on or in the body, with the different body sites having distinct community members. These communities were dominated by four groups of related bacteria, with no one particular group found on all of the body sites of any individual on any given day in this study. In addition, the bacterial community composition at some body sites, such as in the gut, varied considerably between different people. However, each individual's "personalized microbiota" appeared to be relatively stable over time. By defining the composition and variation of the microbiota in healthy individuals, researchers now have a baseline for detecting changes in the microbiota that may be associated with human diseases.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, and Knight R. Bacterial community variation in human body habitats across space and time. Science 326: 1694-1697, 2009.

http://auth.www2.niddk.nih.gov/NR/exeres/E96FC4AD-56D3-4A5A-94D7-073179CD57A1,frameless.htm?NRMODE=Update&WBCMODE=AuthoringReedit

Genetic Risk Factors Associated with Inflammatory Bowel Diseases (IBD) in Children

Researchers have identified five new genetic variations that predispose children to developing IBD. The two major forms of IBD—Crohn's disease and ulcerative colitis—are marked by chronic and destructive inflammation in the intestinal tract. While the precise causes of IBD are unknown, there is a strong genetic component that predisposes individuals to developing these diseases. Previously, nearly 50 genetic risk factors had been identified as associated with IBD. Many of these genetic factors involved components of the immune system that control intestinal inflammation. These risk factors, however, were typically identified in adults with IBD. Since some clinical aspects of IBD in children differ from those in adults, it was important to extend these studies to identify genetic risk factors associated with the development of IBD in pediatric populations.

An international team of researchers has carried out the largest genome-wide association study (GWAS) to date for identifying genetic variants associated with IBD in children. In a GWAS, scientists scan thousands of genomes for genetic variants that are more common in individuals with a disease—such as a form of IBD—than in healthy individuals. In this study comparing children with IBD and healthy children, the researchers identified variants in five new regions of the genome that increase children's risk of developing IBD. One of the most prominent risk factors identified for Crohn's disease was found near the *IL27* gene. This gene codes for a protein that is involved in an immune response previously implicated in the pathogenesis of Crohn's disease. The researchers showed that colon cells from children with Crohn's disease had much lower levels of *IL27* gene activity than cells from healthy individuals, suggesting that the risk variant reduces the amount of protein made from the *IL27* gene. In addition to the five new genetic regions, the pediatric population also has many of the genetic risk factors previously identified in adults with Crohn's disease or ulcerative colitis. This implies that these forms of IBD involve similar biological pathways in adults and children, but that IBD which develops in children may involve some distinct pathways, as well. Defining the genetic variations that predispose individuals to developing IBD enables researchers to gain insight into the causes of these diseases and potentially develop strategies to help detect, treat, and prevent early-onset IBD in children.

Imielinkski M, Baldassano RN, Griffiths A, et al. Common Variants at Five New Loci Associated with Early-Onset Inflammatory Bowel Disease. Nat Genet 41: 1335-1340, 2009.

Mapping Gene Regulatory Sites to Understand Islet Biology and Diabetes

Scientists have developed a new technique for identifying regions of the genome that influence gene activity and generated insight into how a specific sequence variant can affect risk for type 2 diabetes. Regions of the genome called "open chromatin" contain genes currently being used within a cell, as well as regulatory DNA elements that influence the way cells utilize those genes. Using a new technique, the investigators isolated open chromatin from human pancreatic islet cells, which produce insulin and other important hormones, and created a map of these sites in the genome. They hypothesized that islet-specific open chromatin was likely to contain sequences that influence the activity of islet-specific genes and, therefore, sequences that may be associated with diabetes risk. They found that most islet-specific open chromatin sites were in or near genes with known functions in islets. In addition, they compared previously identified type 2 diabetes-associated DNA sequence variants to their map and discovered that a number of disease-associated variants are linked to islet-specific open chromatin sites.

Notably, a variant in the gene *TCF7L2*, which has been consistently associated with type 2 diabetes across diverse ethnic groups, was determined to overlap with an islet-specific open chromatin site. Importantly, the disease-associated variation is not within the part of the gene that codes for a protein, so the type 2 diabetes risk associated with *TCF7L2* cannot be explained by an alteration in the protein encoded by the gene. The scientists then demonstrated that the risk-associated sequence variant was more likely than the non-risk sequence variant to be found in open chromatin, meaning that people with the risk version may produce more of the protein encoded by *TCF7L2*. Indeed, they found that the risk-associated variant can affect gene activity. These results suggest that the risk variant may affect activity of *TCF7L2* by opening the site to allow more of the protein to be made, and provide a potential mechanism for the type 2 diabetes susceptibility.

The islet-specific map generated in this study provides a new tool for understanding the regulation of genes important for islet cell biology and for narrowing genomic locations likely to harbor unidentified sequence variants that influence type 2 diabetes suceptibility. This study also validates a new technique for identifying regions of the genome that regulate gene activity. The technique provides an additional means to move beyond identification of disease-associated sequence variants to an understanding of their influence on disease risk, particularly for variants that do not affect the code for a protein. Determining the mechanism by which genetic factors contribute to diabetes is key to understanding both type 1 and type 2 diabetes, identifying individuals at risk, developing and testing prevention strategies, and generating more personalized interventions for people with or at risk for disease.

Gaulton KJ, Nammo T, Pasquali L, et al. A map of open chromatin in human pancreatic islets. Nat Gen 42: 255-259, 2010

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Identification of a Key Mediator of Fat Cell Development

Scientists used a novel quantitative method to identify molecular regulators needed to form cells with the potential to become fatty tissues in mice and thereby discovered a critical component of the developmental process that generates fatty (adipose) tissues. Significant progress has been made in uncovering the factors and mechanisms that control the process by which mature fat cells (adipocytes) develop from cells with the potential to become adipocytes (preadipocytes) in response to molecular cues. Little is known, however, about how cells become or are maintained as preadipocytes. To identify preadipocyte regulators, the researchers generated new mouse cell lines and evaluated their capacity to form adipocytes. Creation of cell lines with strong propensity to develop into fat cells, as well as lines with little such capacity, allowed the scientists to use a new quantitative molecular tool they developed to identify regulators present at different levels in these different types of cells. They reasoned that factors important for directing cells to be preadipocytes would likely be present at much greater levels in precursors of fat cells compared to precursors of other types of cells or mature fat cells. One factor in particular, called Zfp423, was chosen for further characterization. As one way to test its role in preadipocytes, the scientists engineered high levels of Zfp423 into cells that would not ordinarily have the capacity to become fat cells and looked to see what happened. They found that increased levels of Zfp423 were able to drive these cells to become adipocytes.

The scientists also tested Zfp423's importance with another approach. Using molecular biology techniques, the scientists decreased levels of Zfp423 in preadipocytes and observed that these cells had reduced ability to become adipocytes and had lower levels of other molecular markers characteristic of adipocytes. They demonstrated that Zfp423 is an important regulator of one of these molecular markers—*PPARgamma*, itself another regulator of fat cell development. The researchers also found that Zfp423 plays a key role in production of both major forms of fat in mice (brown fat and white fat). Thus, this important study described a new tool for identifying molecular regulators of cell development and revealed the important role of Zfp423 to the preadiopocyte state. Understanding the formation and properties of fat can help inform strategies to prevent or reduce obesity.

Gupta RK, Arany Z, Seale P, et al. Transcriptional control of preadipocyte determination by Zfp423. Nature 464: 619-623, 2010.

Protein Found That Drives Development of Insulin-producing Cells

Finding ways to reduce or eliminate the burden of injected insulin therapy for people with type 1 diabetes and some with type 2 diabetes is an important goal of diabetes research. One approach to eliminating the dependency on injected insulin is to replenish a person's insulin-producing beta cells. Stem cells or other types of cells that could be reprogrammed to produce insulin may represent a good source of replacement tissue, but to tap their potential it is critical to understand the developmental program that creates a functional beta cell. New research uncovered a key factor necessary for making insulin-producing beta cells in both humans and mice. Previous research had identified a protein that helps trigger embryonic development of pancreatic islets, which contain beta cells and other cell types. Scientists now have found another key protein needed for the subsequent development of distinct islet cell subtypes. Mice lacking the newly identified protein—called Rfx6—can make islets, but these islets do not contain insulin-producing cells. They also fail to make some other hormones normally made by the pancreas. Interestingly, the scientists also found that a rare form of neonatal diabetes is associated with mutations in the human gene that produces the Rfx6 protein, suggesting that Rfx6 plays a critical role in beta cell development in humans as well as mice. Researchers therefore now know they will have to ensure that Rfx6 is present in order to successfully generate beta cells from some other cell type for transplantation into people with diabetes.

Smith SB, Qu H-Q, Taleb N, et al. Rfx6 directs islet formation and insulin production in mice and humans. Nature *463: 775-780, 2010.*

Inexpensive, Generic Drug Improves Blood Glucose Control in People with Type 2 Diabetes

Researchers have discovered that the drug salsalate helped people with type 2 diabetes control their blood glucose levels. Salsalate is an inexpensive, generic anti-inflammatory drug that is chemically similar to aspirin, but causes fewer stomach problems. It has been used safely for decades to treat people with arthritis. Because research is showing that metabolic conditions, including type 2 diabetes, are associated with chronic inflammation, scientists tested whether this anti-inflammatory drug could effectively treat people with type 2 diabetes. In the first phase of the Targeting Inflammation with Salsalate in Type 2 Diabetes (TINSAL-T2D) clinical trial, 108 people were randomly assigned to four different treatment regimens: one group received placebo and three groups received different doses of the drug. All participants continued their regular diabetes treatment regimen during the trial. After 3 months, people taking salsalate had lower blood glucose and triglyceride levels on average compared to people taking placebo. Some participants experienced adverse changes such as increased excretion of protein in the urine and higher levels of LDL (bad) cholesterol. Thus, researchers are conducting a longer, larger trial to further test salsalate—knowledge that is needed to further evaluate the relative benefits and risks of the drug. With more research, salsalate may prove to be an inexpensive way to help treat the millions of people with type 2 diabetes in the U.S.

Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, and Shoelson SE. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 152: 346-357, 2010.

Improving Cord Blood Stem Cell Transplantation for Patients with Various Blood Diseases

A team of researchers developed the first successful laboratory culture system for increasing or expanding the numbers of cord blood stem cells in order to shorten the time necessary for complete engraftment for bone marrow transplantation. Umbilical cord blood is a source of blood-forming cells used in transplants. However, its utility is restricted due to the relatively small number of stem cells in a unit of cord blood. Because of this limitation, compared with a conventional bone marrow transplant, cord blood transplants take longer to fully repopulate all the different types of blood cells in the body. The longer timeframe for engraftment places the patient at increased risk of acquiring life-threatening infections, owing to the inadequate number of white blood cells. For this reason, cord blood is used more often in patients with a small body size, for example children, as they require fewer cells. Patients with larger bodies may have to be transplanted with two or more units of cord blood and still may contend with engraftment times longer than conventional bone marrow transplant.

The investigators took advantage of their knowledge of the "Notch" signaling pathway which stimulates expansion (cell division) of stem cells. A protein was engineered in the laboratory that activates the Notch pathway. The protein was used to stimulate expansion of cord blood stem cells in culture. The presence of the protein resulted in a greater than 100-fold increase in cultured cord blood stem cells compared with cells grown in the absence of the protein.

The researchers then conducted a pilot study of 10 patients with leukemia to begin to assess the safety of infusing cord blood stem cells that had been expanded in the laboratory with this Notch-mediated procedure and to perform an initial evaluation of the engraftment properties of the expanded stem cells. Each patient received two units of cord blood—one unit of non-expanded blood and one containing expanded blood cells that had been expanded with this procedure or two units of non-expanded blood. In this small group of patients, the investigators reported that they did not encounter safety issues. The median time for engraftment using the expanded cells was 16 days versus 26 days when non-expanded units of cord blood were used.

The study's intriguing results suggest that engraftments derived from expanded cells may proceed more rapidly than those derived using conventional (non-expanded) cord blood. These results need to be followed up by a larger study in order to develop statistically significant results.

Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, and Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat Med 16: 232-237, 2010.

Scientists Identify a Potent Inhibitor of Kidney Fibrosis

Researchers have identified the circulating protein serum amyloid P (SAP) as a natural inhibitor of fibrosis during inflammatory injury in the kidney. Using two different models of kidney injury and fibrosis in mice, researchers found that human SAP can potently inhibit fibrosis in this organ. SAP accumulated at sites of injury within the kidney, where it appeared to be associated with injured or dead cells. In the kidney, SAP acts on monocytes and marcophages, specialized white blood cells that are involved in the inflammatory response. SAP suppresses the activity of these inflammatory cells by binding to Fc-gamma receptors on their surfaces. This inhibition of cell activity is dependent on the increased production of the anti-inflammatory protein interleukin-10. SAP has previously been shown to suppress fibrosis in the lung, but through a different mechanism than that seen in the kidney. Taken together, these observations suggest that SAP may have the potential to act as a broad-based anti-fibrotic agent.

The repair of organ and tissue damage is a complex and multistep process, with an initial inflammatory response that attempts to resolve the insult coupled with wound healing and tissue remodeling. Under certain conditions, unrestrained tissue "repair" leads to the excessive deposits of fibrous scar tissue. This process, termed fibrosis, can impair organ function and, left unchecked, lead to organ failure.

This research identifies previously unknown mechanisms of action of SAP in regulating anti-inflammatory activity, and raises the possibility of using SAP or similar agents as a therapy for fibrotic kidney diseases. Subsequent testing in patients who have ongoing fibrotic diseases may determine whether the therapeutic potential seen in these mouse studies can be translated into a novel clinical intervention in patients.

Castaño AP, Lin S-L, Surowy T, Nowlin BT, Turlapati SA, Patel T, et al. Serum Amyloid P Inhibits Fibrosis through Fc-gamma R-Dependent Monocyte-Macrophage Regulation in Vivo. *Sci Transl Med 1: 5ra13, 2009.*

Intestinal Bacteria May Contribute to Developing Obesity and Diabetes

Scientists recently discovered that intestinal bacteria may have an important role in the development of metabolic syndrome, diabetes and obesity. Metabolic syndrome is a constellation of disorders that increases the risk of developing diabetes and cardiovascular disease. Hallmarks include elevated blood sugar, insulin resistance, abnormal blood cholesterol, high blood pressure, fatty liver disease, and obesity – particularly excess abdominal fat. Bacteria that inhabit the digestive tract appear to influence metabolism by affecting the ability to extract energy from food. Furthermore, certain types of intestinal bacteria may play a role in developing obesity, type 2 diabetes, and other aspects of metabolic syndrome. The types of bacteria populating the gut may be determined in part by a protein called Toll-like receptor (TLR) 5. This protein is produced in abundance by cells in the intestinal lining, is important for recognizing microbes, and is part of the innate immune system that can respond to infectious bacteria. Mice lacking TLR5 develop intestinal infections and gain weight, leading scientists to believe that the protein may also influence metabolism, potentially by altering normal gut bacteria.

To further explore the role of intestinal bacteria and TLR5 in developing metabolic syndrome, scientists generated mice that do not produce TLR5 protein. These animals weighed about 20 percent more than normal mice by 20 weeks of age, consumed about 10 percent more food, and produced more body fat compared to their normal counterparts. The TLR5-deficient mice also developed elevated cholesterol levels, increased blood pressure, and insulin resistance. When fed a high-fat diet for eight weeks, both normal and TLR5-deficient mice gained weight but, unlike normal mice, TLR5-deficient animals developed type 2 diabetes and fatty livers. One possible explanation was that TLR5-deficient mice ate a greater quantity of high-fat food. When scientists restricted the amount of high-fat food so that TLR5-deficient mice and normal mice at the same quantity, the TLR5-deficient animals did not become obese, but were insulin-resistant. The investigators thought that the metabolic differences observed between the two strains of mice were due to changes in intestinal bacterial populations resulting from the loss of TLR5. Thus, the researchers compared the gut bacteria between the normal and TLR5-deficient mice and uncovered differences in levels of over 100 types of bacteria. To assess whether the changes in bacteria might be causing the metabolic symptoms, the scientists collected intestinal bacteria from TLR5-deficient mice and transplanted these into “germ-free” mice raised in a bacteria-free environment. Similar to TLR5-deficient mice, the mice who received the transplanted bacteria increased their food consumption, developed insulin resistance, and became obese.

This study demonstrates that bacteria in the gut may contribute to changes in appetite and metabolism. Excess calorie consumption along with the resulting obesity and development of type 2 diabetes could possibly be driven, at least in part, by alterations in intestinal bacteria populations due to compromised bacterial via alterations in biological pathways involving TLR5. Understanding how gut bacteria interact with the intestine could provide a means of modulating eating behavior as well as preventing metabolic syndrome development.

Vijay-Kumar, M, Aitken JD, Carvalho FA, et al. Metabolic Syndrome and Altered Gut Microbiota in Mice Lacking Toll-Like Receptor 5. Science 328: 228-231, 2010.